Novel Substituted Imidazo[2,1-*b*][1,3,4]Thiadiazole Derivatives: Synthesis, Characterization, Molecular Docking Study, and Investigation of Their *In Vitro* Antifungal Activities

Mustafa Er,[a]* Hakan Tahtaci,[a] Tuncay Karakurt,[b] and Abdurrahman Onaran[c]

[a]Department of Chemistry, Faculty of Science, Karabuk University, 78050, Karabuk, Turkey
[b]Department of Chemical Engineering, Faculty of Engineering and Architecture, Ahi Evran University, 40100, Kırşehir, Turkey
[c]Department of Plant Protection, Faculty of Agriculture, Gaziosmanpasa University, 60250, Tokat, Turkey
*E-mail: mustafaer@karabuk.edu.tr

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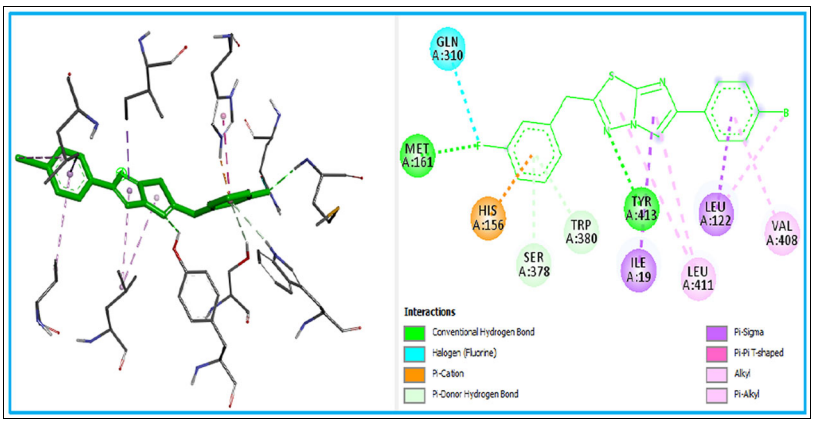

In this study, a new series of substituted imidazo[2,1-*b*][1,3,4]thiadiazole derivatives were synthesized. To this end, first 2-amino-1,3,4-thiadiazole derivatives (compounds **2a** and **2b**), the starting materials, were synthesized with high yields (82% and 79%, respectively). Then imidazo[2,1-*b*][1,3,4]thiadiazole derivatives (**4**–**16**), the target compounds, were synthesized from reactions of 2-amino-1,3,4-thiadiazole derivatives (**2a** and **2b**) with 2-bromoacetophenone derivatives (**3a**–**3i**) (in yields of 52% to 71%). All of the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, Fourier transform infrared, elemental analysis, mass spectroscopy, and X-ray diffraction analysis (compounds **4**–**12**, **14**, and **15**) techniques. *In vitro* antifungal activity tests were performed for all of the synthesized compounds. Inhibition zones, percentage of inhibition, minimum fungicidal activity, minimum inhibitory concentration, and lethal dose values of the target compounds were determined against some plant pathogens. According to the results of the biological activity tests, all of the synthesized compounds showed moderate to high levels of antifungal activity. Theoretical calculations were performed to support the experimental results. The geometric parameters of selected compounds (**5**, **6**, and **8**) were optimized using the density functional theory B3LYP/6-31G(d) method in the Gaussian 09W package program, and the frontier molecular orbitals (highest occupied molecular orbital–lowest unoccupied molecular orbital) were calculated theoretically. Finally, molecular docking studies were performed for antifungal activity studies of the selected compounds and to determine whether or not these compounds could be inhibitor agents for the 2RKV protein structure.

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INTRODUCTION

Among the most commonly used heterocyclic compounds in pharmaceutics and clinical drugs are imidazole, 1,3,4-thiadiazole, and their derivatives. In recent years, synthesis and characterization studies of bioactive compounds such as imidazole and 1,3,4-thiadiazole have been steadily increasing [1–5]. The synthesis of compounds containing these bioactive groups together with various biological activities has received much interest as well [6–10].

These heterocyclic systems, in which imidazole and 1,3,4-thiadiazole rings are fused to each other with a bridgehead nitrogen atom, are referred to as imidazo[2,1-*b*][1,3,4]thiadiazoles [11]. Imidazo[2,1-*b*][1,3,4]thiadiazoles and their derivatives are widely used in medicine, pharmaceutics, and pharmaceutical chemistry because of their numerous biological activities, including antimicrobial [12,13], antifungal [14], antibacterial [15–17], anti-inflammatory,[18–20] antitubercular [21–24], analgesic [25], and diuretic [26] activities.

Imidazo[2,1-*b*][1,3,4]thiadiazole and its derivatives are known to have high anticancer activity. As is known, cancer is a disease in which cells divide uncontrollably and invade surrounding tissues. There are different

assumptions regarding the causes of this disease. According to one hypothesis, TGFβ receptors cause metastasis because of loss of heterozygosity. The signaling pathway of the TGFβ receptors must be inhibited to stop the metastasis of cancer cells. The intracellular part of TGFβ receptors is the starting point of the signaling pathway, which supports metastasis [27–31].

In this part of the TGFβ receptor, phosphate groups in adenosine triphosphate (ATP) are transferred to the TGFβ receptor, which constitutes the first stage of the signal that triggers metastasis. Since the structure of imidazo[2,1-*b*][1,3,4]thiadiazole derivatives is similar to that of ATP, they stop the phosphorylation reaction by binding to TGFβ receptors instead of ATP molecules, thereby creating an anticancer effect [32,33].

In addition to anticancer activity, imidazo[2,1-*b*][1,3,4] thiadiazole derivatives have antimicrobial activity as well. Pathogens gain resistance to antimicrobial drugs over time, which leads to new problems. For this reason, it is necessary to design and synthesize new antimicrobial drugs to protect against epidemics [34].

In light of the aforementioned important data, this study aims to synthesize imidazo[2,1-*b*][1,3,4]thiadiazole derivatives with potential biological activities, characterize them *via* various spectroscopic methods, and investigate the antifungal activities of these compounds.

Another purpose of the study is to calculate the optimized structures and highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energy values of the most actives compounds (**5**, **6**, and **8**) among the synthesized compounds using the density functional theory (B3LYP) [35,36] theory with 6-31G(d) basis set, which is a quantum chemical calculation, in the Gaussian 09 program [37]. The study further aims to determine whether or not these optimized compounds could be candidate inhibitor agents for the 2RKV *Fusarium graminearum* protein structure using the molecular docking simulation method.

RESULTS AND DISCUSSION

Chemistry. In this study, 2-amino-1,3,4-thiadiazole derivatives (**2a** and **2b**) were first synthesized. 2-Amino-1,3,4-thiadiazole derivatives (**2a** and **2b**) were obtained from reactions of nitrile compounds (**1a** and **1b**) with thiosemicarbazide in trifluoroacetic acid with high yields (82% and 79%, respectively). The nitrile derivatives (**1a** and **1b**) used as starting materials to synthesize imidazo[2,1-*b*][1,3,4]thiadiazole derivatives (**4–16**) were commercially obtained, while 2-amino-1,3,4-thiadiazole derivatives (**2a** and **2b**) were obtained as specified in the literature [38,39].

The sharp absorption bands observed around 2220–2260 cm^{-1} that belong to the nitrile groups (C≡N) disappeared in the IR spectra of 2-amino-1,3,4-thiadiazole derivatives (**2a**,**b**). It was observed that symmetric and asymmetric absorption bands appeared instead as two separate bands belonging to the $–NH_2$ group of 2-amino-1,3,4-thiadiazole derivatives (**2a** and **2b**) in the 3271–3112 cm^{-1} range, which indicates that these compounds were formed.

The $–NH_2$ group proton signals of compounds **2a** and **2b** bonded to the 1,3,4-thiadiazole ring from the C_2 position in ^1H NMR spectra were observed as a singlet corresponding to two protons in the range of 7.14–7.03 ppm. Proton peaks belonging to the $–NH_2$ group of these compounds (**2a** and **2b**) disappeared as a result of proton-deuterium exchange performed with D_2O.

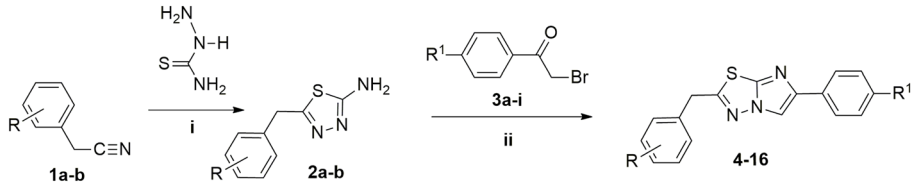
The structures of compounds **2a** and **2b** were confirmed by the ^{13}C NMR spectra. In the ^{13}C NMR spectra of these compounds, C_2 carbon signals belonging to the thiadiazole ring were observed at 157.12 and 156.39 ppm for compounds **2a** and **2b**, respectively. The C_5 carbon signals of the same ring were recorded as 169.33 and 169.78 ppm, respectively. In the ^{13}C NMR spectra, it was seen that resonance values belonging to carbons of the thiadiazole ring of these compounds were highly compatible with this type of compound in the literature [12,40]. Other ^{13}C NMR spectral data of the molecule verified the suggested structure.

In the final step, imidazo[2,1-*b*][1,3,4]thiadiazole derivatives (**4–16**), the target compounds, were synthesized from reactions of 2-amino-1,3,4-thiadiazole derivatives (**2a** and **2b**) with 2-bromoacetophenone derivatives (**3a–3i**) in absolute ethyl alcohol. The synthetic pathway is shown in Scheme 1.

The most important evidence for the formation of imidazo[2,1-*b*][1,3,4]thiadiazole derivatives in the IR spectra of these compounds is the disappearance of symmetric and asymmetric absorption bands belonging to the $–NH_2$ group in 2-amino-1,3,4-thiadiazole derivatives (**2a** and **2b**) observed in the 3271–3112 cm^{-1} range. Furthermore, the $–NH_2$ group proton signals observed in the 7.14–7.03 ppm range in the initial compounds (**2a** and **2b**) disappeared in the ^1H NMR spectra of these compounds. Instead, C_5–H signals were observed as a singlet corresponding to one proton in the 8.93–8.50 ppm range, which indicates ring cyclization and is the most important evidence for the formation of imidazo[2,1-*b*][1,3,4]thiadiazole derivatives (**4–16**). These values are consistent with those of similar studies in the literature [12,41].

Further, important evidence indicating ring cyclization in imidazo[2.1-*b*][1,3,4]thiadiazole derivatives (**4–16**) is the observation of signals in the ranges of 113.52–109.61 ppm and 146.18–143.14 ppm in the ^{13}C NMR spectra,

Scheme 1. The synthetic route for the synthesis of substituted imidazo[2,1-*b*][1,3,4]thiadiazole derivatives (**4**–**16**). Reagent and conditions: i: trifluoroacetic acid, 60°C, reflux, 3 h; ii: absolute ethanol, reflux, 12–16 h. [Color figure can be viewed at wileyonlinelibrary.com]



R: 3 -F for **4-12**
R: 4 -NO_2 for **13-16**

Comp.	R^1	Comp.	R^1
4	-H	12	
5	-Br		
6	-Cl	13	-H
7	-F	14	-CN
8	$-OCH_3$	15	-Ph
9	$-NO_2$	16	
10	-CN		
11	-Ph		

corresponding to C_5 and C_6 carbons, respectively. Peaks belonging to C_2 and C_8 carbons in these compounds were observed in the 165.15–163.26 ppm and 145.32–140.89 ppm ranges, respectively. Other ^{1}H NMR and ^{13}C NMR spectral data of these compounds are presented in the experimental part of this study in detail. The ^{1}H NMR and ^{13}C NMR spectra of all synthesized compounds are given in the Supporting Information.

The mass spectra belonging to the synthesized compounds were observed as expected with molecular ion peaks.

In addition, the structures of compounds **4**–**12**, **14**, and **15** were confirmed by X-ray diffraction analysis. The crystal structures and crystallographic data of these compounds are shown in Figure 1 and Table 1, respectively. Rotational disorder was observed for the F atoms of crystals **5**, **8**, and **12**. Bond lengths, bond angles, and packing structures of compounds **4**–**12**, **14**, and **15** are given in the Supporting Information.

In vitro antifungal activity studies. *In vitro* antifungal activity values of all synthesized compounds were determined. Inhibition zones, percentage of inhibition (PI), minimum fungicidal activity (MFC), minimum inhibitory concentration (MIC), and lethal dose (LD_{50}) values of compounds were determined against some plant pathogens (Tables 2 and 3 and Figs 2 and 3). Varying activities were observed for 500 and 1000 µg/mL doses of all used compounds. The activity rate increased with increasing dose. The fungus that was most sensitive to the compounds was *Fusarium oxysporum* f.sp. *melonis*, followed by *Alternaria solani*, *Monilia fructigena*, *Verticillium dahliae*, *Sclerotinia sclerotiorum*, and *Rhizoctonia solani*. Fungicides with 50% Manep, 80% Thiram, and 80% Captan active substances, used as a positive control, showed activity against all fungi at the recommended doses. Some compounds showed the same degree of effect as the positive control (Table 2 and Figs 2 and 3). Dimethyl sulfoxide (DMSO), used as the negative control, showed no antifungal activity. PI values were determined considering the mycelium development in the negative control. The PI value of test fungi for 1000 µg/mL varied from 55% to 100% for *M. fructigena*, from 0% to 72% for *R. solani*, from 52% to 88% for *A. solani*, from 52% to 86% for *V. dahliae*, from 0% to 68% for *S. sclerotiorum*, and from 65% to 100% for *F. oxysporum* f.sp. *melonis*. MFC, MIC, and LD_{50} values of compounds against the fungi were also calculated (Table 3). MFC values varied from >250 to >1000 µg/mL. These values varied depending on activities of compounds against test fungi. Similarly, MIC values varied depending on reactions of test fungi against compounds. These activity values varied between 31.25 and 1000 µg/mL for MIC (Table 3). LD_{50} values varied between doses of 253 and 1235 µg/mL. These values were calculated to be 397 to 986 µg/mL for *M. fructigena*, 561 to 1235 µg/mL for *R. solani*, 367 to 880 µg/mL for *A. solani*, 610 to 1062 µg/mL for *V. dahliae*, 728 to 1173 µg/mL for *S. sclerotiorum*, and 253 to 634 µg/mL for *F. oxysporum* f.sp. *melonis*.

Molecular docking studies. Molecular docking studies of compounds **5**, **6**, and **8** were performed using the AutoDock Vina [42] program. AutoDock Tools were used for creating docking data entry files, and receptor–ligand interactions were demonstrated with Discover

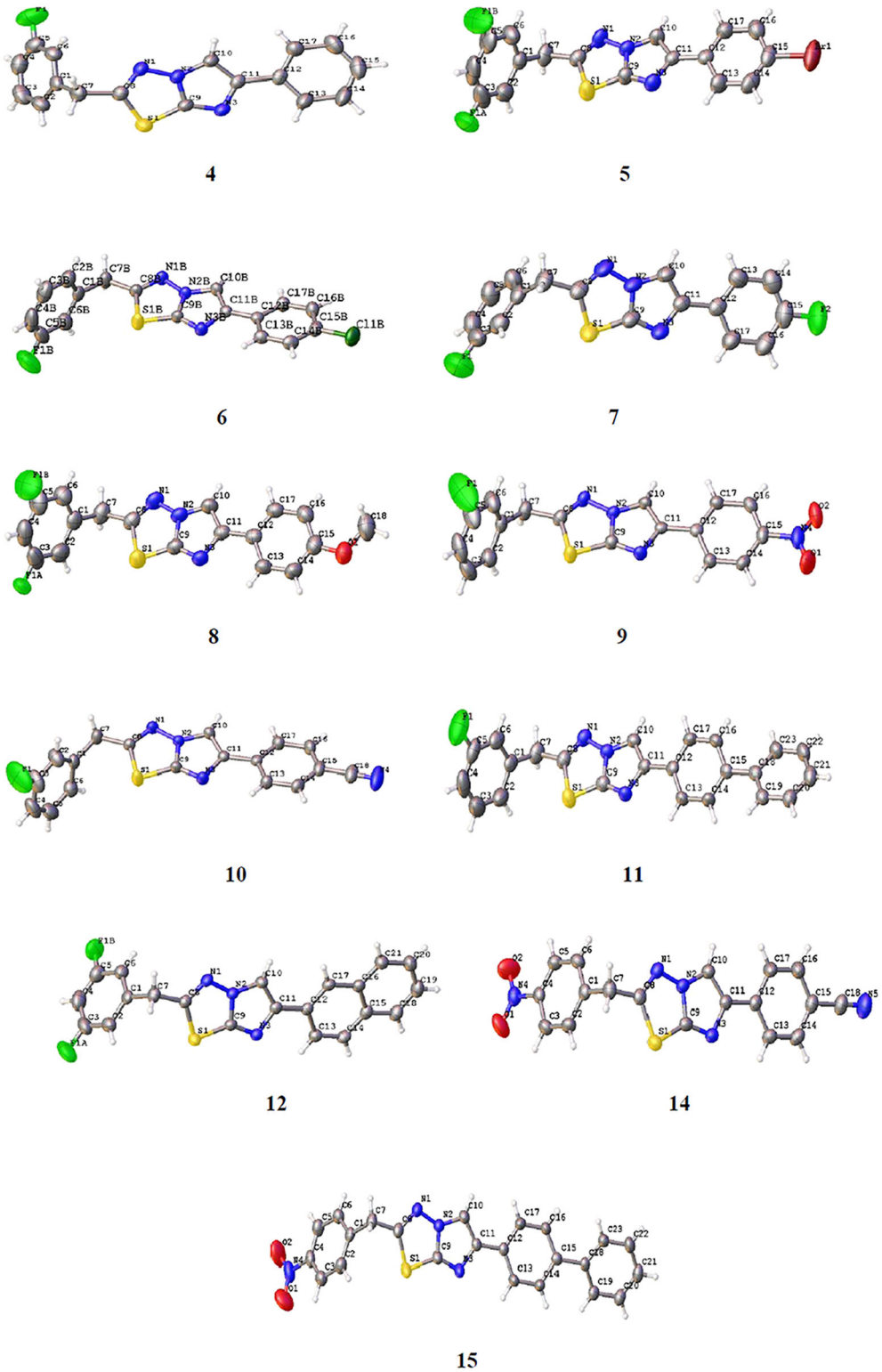


Figure 1. The crystal structures of compounds **4**–**12**, **14**, and **15**. [Color figure can be viewed at wileyonlinelibrary.com]

Studio Visualizer 2017 software [43]. Molecular docking studies were conducted using *Coenzyme A* with a resolution of 1.6 A and *T-2 mycotoxin* (ZBA) and X-ray crystal structures of trichothecene 3-*O*-acetyltransferase (PDB ID: 2RKV) in complex form. Before proceeding to calculations, the crystal structures of 2RKV and all ligand

Table 1

Crystallographic data for compounds **4**–**12**, **14**, and **15**.

Compounds	**4**	**5**	**6**	**7**	**8**	**9**
Molecular formula	$C_{17}H_{12}FN_3S$	$C_{17}H_{11}BrFN_3S$	$C_{17}H_{11}N_3FSCl$	$C_{17}H_{11}F_2N_3S$	$C_{18}H_{14}FN_3OS$	$C_{17}H_{11}FN_4O_2S$
Molecular weight	309.36	388.25	343.80	327.35	339.37	354.36
Temperature (K)	293(2)	293(2)	293(2)	293(2)	293(2)	293(2)
Crystal system	Monoclinic	Monoclinic	Triclinic	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/n$	P-1	C2/c	$P2_1/n$	$P2_1/c$
a (Å)	5.7382(4)	10.6616(6)	5.5541(3)	28.323(6)	10.9799(7)	20.8893(16)
b (Å)	14.0096(10)	5.6746(3)	16.4702(10)	5.5819(10)	5.6707(3)	5.6676(4)
c (Å)	17.9184(14)	26.2725(14)	17.5949(10)	20.176(5)	25.6008(18)	13.8951(12)
α (°)	90	90	106.021(2)	90	90	90
β (°)	91.503(2)	90.971(2)	94.214(2)	110.153(7)	90.240(2)	107.161(3)
γ (°)	90	90	93.901(2)	90	90	90
Volume ($Å^3$)	1439.96(18)	1589.27(15)	1536.27(15)	2994.5(11)	1593.99(17)	1571.8(2)
Z	4	4	4	8	4	4
ρcalcg/cm^3	1.427	1.618	1.486	1.452	1.410	1.497
μ/mm^{-1}	0.235	2.729	0.396	0.239	0.223	0.236
F(000)	640.0	772.0	704.0	1344.0	700.0	728.0
Crystal size (mm^3)	0.15 × 0.12 × 0.11	0.17 × 0.13 × 0.11	0.16 × 0.13 × 0.11	0.21 × 0.17 × 0.13	0.19 × 0.16 × 0.14	0.21 × 0.17 × 0.15
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for compound data collection (°)	5.816 to 56.676	5.97 to 49.988	6.002 to 56.694	6.082 to 49.986	6.034 to 56.6	6.124 to 50
Index ranges	$-6 \le h \le 7$, $-18 \le k \le 18$, $-23 \le l \le 23$	$-12 \le h \le 12$, $-6 \le k \le 6$, $-31 \le l \le 31$	$-7 \le h \le 7$, $-21 \le k \le 21$, $-23 \le l \le 23$	$-33 \le h \le 33$, $-6 \le k \le 6$, $-23 \le l \le 23$	$-14 \le h \le 12$, $-7 \le k \le 7$, $-34 \le l \le 33$	$-24 \le h \le 24$, $-6 \le k \le 6$, $-16 \le l \le 16$
Reflections collected	32661	37624	75812	44365	31894	40734
Independent reflections	3589 [R_{int} = 0.0328, R_{sigma} = 0.0177]	2748 [R_{int} = 0.0676, R_{sigma} = 0.0271]	7655 [R_{int} = 0.0548, R_{sigma} = 0.0317]	2636 [R_{int} = 0.0414, R_{sigma} = 0.0173]	3952 [R_{int} = 0.0574, R_{sigma} = 0.0387]	2671 [R_{int} = 0.0698, R_{sigma} = 0.0266]
Data/restraints/parameters	3589/0/199	2748/0/217	7655/0/415	2636/0/209	3952/0/227	2671/0/226
Goodness-of-fit on F^2	1.044	1.099	1.060	1.072	1.077	1.046
Final R indexes [$I \ge 2\sigma(I)$]	R_1 = 0.0634, wR_2 = 0.1638	R_1 = 0.0616, wR_2 = 0.1134	R_1 = 0.0615, wR_2 = 0.1527	R_1 = 0.0842, wR_2 = 0.2293	R_1 = 0.0662, wR_2 = 0.1556	R_1 = 0.0471, wR_2 = 0.1056
Final R indexes [all data]	R_1 = 0.0839, wR_2 = 0.1876	R_1 = 0.0914, wR_2 = 0.1345	R_1 = 0.0937, wR_2 = 0.1739	R_1 = 0.0994, wR_2 = 0.2521	R_1 = 0.1128, wR_2 = 0.1785	R_1 = 0.0606, wR_2 = 0.1161
Largest diff. peak/hole/e $Å^{-3}$	0.80/−0.40	0.40/−0.51	1.17/−0.40	1.53/−0.39	0.34/−0.23	0.26/−0.25
CCDC	1538525	1538527	1538532	1538529	1538524	1538530

Table 1

Crystallographic data for compounds **4**–**12**, **14**, and **15**.

Compounds	**10**	**11**	**12**	**14**	**15**
Molecular formula	$C_{18}H_{11}FN_4S$	$C_{23}H_{16}FN_3S$	$C_{21}H_{14}FN_3S$	$C_{18}H_{11}N_5O_2S$	$C_{23}H_{16}N_4O_2S$
Molecular weight	334.37	385.45	359.42	361.38	412.46
Temperature (K)	293(2)	293(2)	293(2)	293(2)	293(2)
Crystal system	Monoclinic	Orthorhombic	Triclinic	Monoclinic	Monoclinic
Space group	$P2_1/c$	Pbca	P-1	$P2_1/n$	$P2_1/c$
a (Å)	20.2694(14)	11.2225(6)	5.7253(4)	7.1195(12)	21.2749(18)
b (Å)	5.6325(4)	8.2232(5)	9.2911(7)	16.646(3)	10.7811(9)
c (Å)	14.1978(10)	39.585(2)	16.4699(13)	13.750(2)	8.2745(8)
α (°)	90	90	74.940(3)	90	90
β (°)	100.582(2)	90	80.765(3)	95.134(6)	91.196(3)
γ (°)	90	90	86.688(3)	90	90
Volume ($Å^3$)	1593.36(19)	3653.1(4)	834.93(11)	1623.0(5)	1897.5(3)
Z	4	8	2	4	4
ρcalcg/cm^3	1.394	1.402	1.426	1.479	1.444
μ/mm^{-1}	0.220	0.201	0.214	0.224	0.200
F(000)	688.0	1600.0	370.0	744.0	856.0
Crystal size (mm^3)	0.19 × 0.16 × 0.14	0.16 × 0.14 × 0.1	0.15 × 0.13 × 0.11	0.23 × 0.19 × 0.17	0.16 × 0.13 × 0.11
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for compound data collection (°)	6.484 to 56.628	6.176 to 56.55	5.772 to 56.694	5.95 to 56.77	6.466 to 56.716
Index ranges	$-27 \le h \le 27$, $-7 \le k \le 7$, $-18 \le l \le 18$	$-14 \le h \le 12$, $-10 \le k \le 10$, $-52 \le l \le 51$	$-7 \le h \le 7$, $-12 \le k \le 12$, $-21 \le l \le 21$	$-9 \le h \le 9$, $-22 \le k \le 22$, $-18 \le l \le 18$	$-28 \le h \le 28$, $-14 \le k \le 14$, $-11 \le l \le 11$
Reflections collected	39456	36789	38134	37476	50404
Independent reflections	3748 [R_{int} = 0.0325, R_{sigma} = 0.0171]	4399 [R_{int} = 0.0659, R_{sigma} = 0.0556]	4151 [R_{int} = 0.0343, R_{sigma} = 0.0185]	3977 [R_{int} = 0.0417, R_{sigma} = 0.0212]	4665 [R_{int} = 0.0445, R_{sigma} = 0.0223]
Data/restraints/parameters	3748/0/218	4399/0/254	4151/0/245	3977/0/235	4665/0/271
Goodness-of-fit on F^2	1.088	1.057	1.043	1.079	1.069
Final R indexes [$I \ge 2\sigma$ (*I*)]	R_1 = 0.0609, wR_2 = 0.1069	R_1 = 0.0841, wR_2 = 0.2060	R_1 = 0.0473, wR_2 = 0.1054	R_1 = 0.0495, wR_2 = 0.1032	R_1 = 0.0533, wR_2 = 0.1129
Final R indexes [all data]	R_1 = 0.0716, wR_2 = 0.1135	R_1 = 0.1470, wR_2 = 0.2272	R_1 = 0.0580, wR_2 = 0.1142	R_1 = 0.0654, wR_2 = 0.1155	R_1 = 0.0707, wR_2 = 0.1261
Largest diff. peak/hole/e $Å^{-3}$	0.27/−0.26	0.75/−0.38	0.33/−0.26	0.23/−0.29	0.33/−0.37
CCDC	1538526	1538523	1538528	1538531	1538533

Table 2

Inhibition zones of compounds **4–16** against plant pathogenic fungi.

	Mean zone of inhibition (mm)[a]											
	Monilia fructigena		*Rhizoctonia solani*		*Alternaria solani*		*Verticillium dahliae*		*Sclerotinia sclerotiorum*		*Fusarium oxysporum* f.sp. *melonis*	
	Doses (μg/mL)		Doses (μg/mL)		Doses (μg/mL)		Doses (μg/mL)		Doses (μg/mL)		Doses (μg/mL)	
Compounds	500	1000	500	1000	500	1000	500	1000	500	1000	500	1000
C−	0 ± 0,00	0 ± 0,00	0 ± 0,00	0 ± 0,00	0 ± 0,00	0 ± 0,00	0 ± 0,00	0 ± 0,00	0 ± 0,00	0 ± 0,00	0 ± 0,00	0 ± 0,00
4	9 ± 0,57	16 ± 0,86	0 ± 0,00	0 ± 0,00	10 ± 0,45	15 ± 0,82	7 ± 1,04	13 ± 0,65	6 ± 0,97	10 ± 1,16	12 ± 0,73	18 ± 0,79
5	10 ± 0,19	22 ± 0,91	0 ± 0,00	7 ± 0,81	9 ± 0,60	15 ± 1,09	5 ± 0,40	13 ± 0,79	3 ± 1,24	10 ± 0,66	11 ± 0,34	15 ± 1,66
6	11 ± 0,90	21 ± 1,26	0 ± 0,00	6 ± 0,75	10 ± 0,80	14 ± 0,97	7 ± 0,52	12 ± 0,63	0 ± 0,00	0 ± 0,00	9 ± 0,82	21 ± 0,63
7	9 ± 1,06	15 ± 0,44	0 ± 0,00	5 ± 0,69	10 ± 0,46	18 ± 1,12	4 ± 0,55	12 ± 1,21	4 ± 0,36	9 ± 0,75	16 ± 0,85	19 ± 0,95
8	11 ± 0,67	22 ± 1,35	0 ± 0,00	2 ± 0,88	9 ± 0,39	17 ± 1,21	8 ± 0,47	16 ± 0,80	3 ± 0,45	11 ± 0,94	11 ± 0,97	22 ± 1,16
9	10 ± 0,78	16 ± 0,26	4 ± 1,20	12 ± 0,94	11 ± 1,29	22 ± 0,88	9 ± 0,72	14 ± 0,39	6 ± 0,72	17 ± 1,99	11 ± 0,58	18 ± 1,00
10	9 ± 0,76	15 ± 0,77	4 ± 0,78	10 ± 1,48	8 ± 1,12	13 ± 0,79	5 ± 0,38	11 ± 0,19	6 ± 1,74	13 ± 0,76	10 ± 0,75	19 ± 0,88
11	8 ± 0,89	17 ± 0,55	5 ± 0,80	10 ± 1,24	9 ± 0,84	14 ± 0,58	6 ± 1,72	13 ± 0,96	5 ± 0,80	11 ± 0,60	13 ± 0,74	20 ± 1,30
12	6 ± 1,08	13 ± 1,41	3 ± 1,13	9 ± 1,19	8 ± 0,68	15 ± 1,07	9 ± 1,10	12 ± 0,47	0 ± 0,00	6 ± 0,95	11 ± 1,17	22 ± 0,78
13	7 ± 0,79	12 ± 0,86	6 ± 1,20	11 ± 1,59	9 ± 1,57	16 ± 0,83	6 ± 1,26	12 ± 1,62	0 ± 0,00	6 ± 0,73	13 ± 0,62	25 ± 0,49
14	9 ± 0,96	13 ± 0,92	5 ± 1,56	9 ± 1,43	7 ± 0,89	15 ± 0,75	8 ± 1,33	13 ± 0,90	4 ± 0,71	11 ± 0,50	9 ± 0,47	17 ± 0,75
15	10 ± 1,06	14 ± 1,01	5 ± 1,14	10 ± 0,29	10 ± 0,29	19 ± 0,85	9 ± 0,49	18 ± 0,87	5 ± 0,69	11 ± 0,77	9 ± 0,51	19 ± 0,69
16	7 ± 0,76	12 ± 0,55	12 ± 0,85	18 ± 1,05	10 ± 0,96	21 ± 1,40	8 ± 1,22	15 ± 0,98	5 ± 0,34	11 ± 1,05	10 ± 0,46	21 ± 0,57
^{1}C+	18 ± 0,77	18 ± 0,77	18 ± 1,24	18 ± 1,24	24 ± 0,64	24 ± 0,64	15 ± 0,67	15 ± 0,67	22 ± 1,23	22 ± 1,23	14 ± 1,65	14 ± 1,65
^{2}C+	23 ± 0,42	23 ± 0,42	21 ± 1,00	21 ± 1,00	25 ± 1,13	25 ± 1,13	20 ± 0,88	20 ± 0,88	25 ± 0,06	25 ± 0,06	25 ± 1,36	25 ± 1,36
^{3}C+	24 ± 1,01	24 ± 1,01	18 ± 2,17	18 ± 2,17	25 ± 0,77	25 ± 0,77	16 ± 1,10	16 ± 1,10	16 ± 0,55	16 ± 0,55	24 ± 0,81	24 ± 0,81

Inhibition zone (IZ) ± standard deviation (SD); $^{1, 2, 3}$C+: Manep (1), Thiram (2), and Captan (3) positive control; C−: negative control.
[a]Mean of three assays.

Table 3

Antifungal activity values (MFC, MIC, and LD_{50}) of target compounds (**4**–**16**).

Compounds	Values	MFC, MIC, and LD_{50} values (μg/mL)					
		Mf	Rs	As	Vd	Ss	FOM
4	MFC	>1000	>1000	>1000	>1000	>1000	>500
	MIC	250	>1000	125	250	250	62.5
	LD_{50}	722	ND	665	921	1133	407
5	MFC	>500	>1000	>1000	>1000	>1000	>1000
	MIC	125	>500	125	500	500	125
	LD_{50}	446	ND	716	953	1173	598
6	MFC	>500	>1000	>1000	>1000	>1000	>1000
	MIC	125	>500	62.5	250	>1000	125
	LD_{50}	470	ND	707	1027	ND	525
7	MFC	>1000	>1000	>1000	>1000	>1000	>250
	MIC	250	>500	125	500	500	31.25
	LD_{50}	735	ND	592	1048	1161	253
8	MFC	>500	>1000	>1000	>1000	>1000	>500
	MIC	62,5	>500	62.5	250	500	62.5
	LD_{50}	397	ND	622	714	1070	387
9	MFC	>1000	>1000	>500	>1000	>1000	>500
	MIC	125	500	31.25	125	250	62.5
	LD_{50}	627	1009	367	787	728	485
10	MFC	>100	>1000	>1000	>1000	>1000	>500
	MIC	125	>250	125	500	250	125
	LD_{50}	726	1170	880	1062	902	538
11	MFC	>1000	>1000	>1000	>1000	>1000	>500
	MIC	250	>250	125	250	500	31.25
	LD_{50}	694	1080	767	931	1100	395
12	MFC	>1000	>1000	>1000	>1000	>1000	>500
	MIC	250	>250	125	250	1000	31.25
	LD_{50}	889	1235	739	872	ND	345
13	MFC	>1000	>1000	>1000	>1000	>1000	>250
	MIC	250	>250	125	500	1000	31.25
	LD_{50}	986	1055	681	990	ND	275
14	MFC	>1000	>1000	>1000	>1000	>1000	>1000
	MIC	250	>250	125	250	500	125
	LD_{50}	803	1166	830	889	1086	634
15	MFC	>1000	>1000	>500	>1000	>1000	>1000
	MIC	>125	>250	31.25	125	500	125
	LD_{50}	693	1094	499	610	1062	580
16	MFC	>1000	1000	>500	>1000	>1000	>500
	MIC	250	125	31.25	125	500	62.5
	LD_{50}	916	561	420	749	1039	482
^{1}C+	MFC	>2000	>2000	2000	>2000	2000	>2000
	MIC	125	250	62.5	500	62.5	125
	LD_{50}	1188	1388	604	1766	844	1682
^{2}C+	MFC	3000	3000	3000	3000	3000	3000
	MIC	62.5	>62.5	31.25	>31.25	31.25	62.5
	LD_{50}	843	1250	649	1377	615	679
^{3}C+	MFC	2000	>2000	>1000	>2000	>2000	2000
	MIC	31.25	125	>31.25	62.5	250	250
	LD_{50}	572	1294	510	1534	1497	927

ND, not determined; LD_{50}, lethal dose; MFC, minimum fungicidal concentration; MIC, minimum inhibitory concentration; C+: Manep (1), Thiram (2), and Captan (3) positive control; Mf, *Monilia fructigena*; Rs, *Rhizoctonia solani*; As, *Alternaria solani*; Vd, *Verticillium dahliae*; Ss, *Sclerotinia sclerotiorum*; FOM, *Fusarium oxysporum* f.sp. *melonis*.

compounds were prepared using protein and ligand preparation wizards in the PyRx package [44]. Prior to the docking process, first water and heteroatoms other than the natural ligand (ZBA) were removed, and hydrogen atoms and Gasteiger charges were added to the 2RKV protein structure.

Molecular docking studies were performed to see possible bonding modes of the synthesized compounds. *Coenzyme A* and *T-2 mycotoxin* (ZBA, natural ligand) and X-ray crystal structures of trichothecene 3-*O*-acetyltransferase were used in the calculations. The biological activity test results showed that compounds **5**,

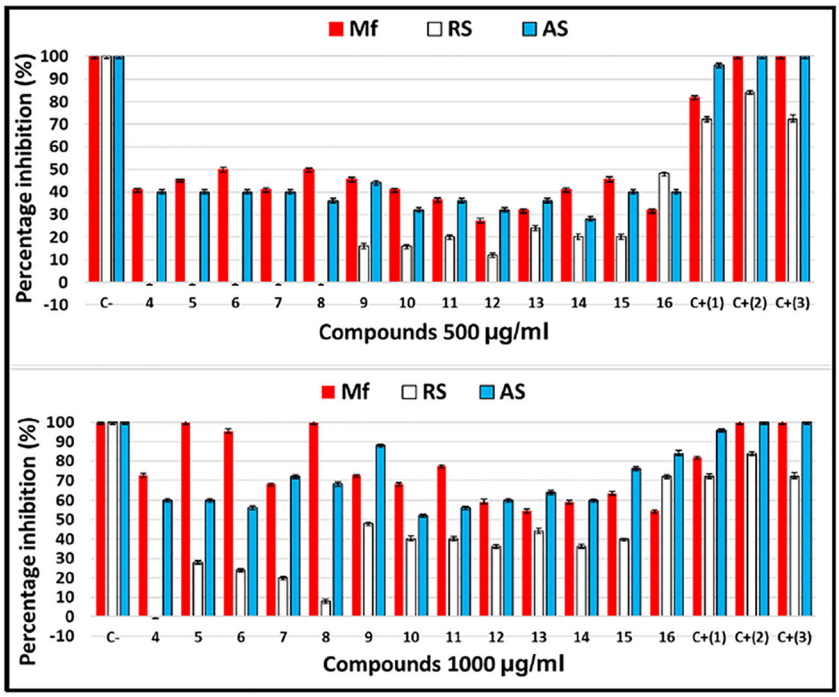


Figure 2. Percentage inhibition of compounds against *M. fructigena* (Mf), *R. solani* (RS), and *A. solani* (AS) for 500 and 1000 µg/mL; C+: Manep (1), Thiram (2), and Captan (3) positive control; C−: negative control. According to negative control, mycelial growth was calculated percentage inhibition values. [Color figure can be viewed at wileyonlinelibrary.com]

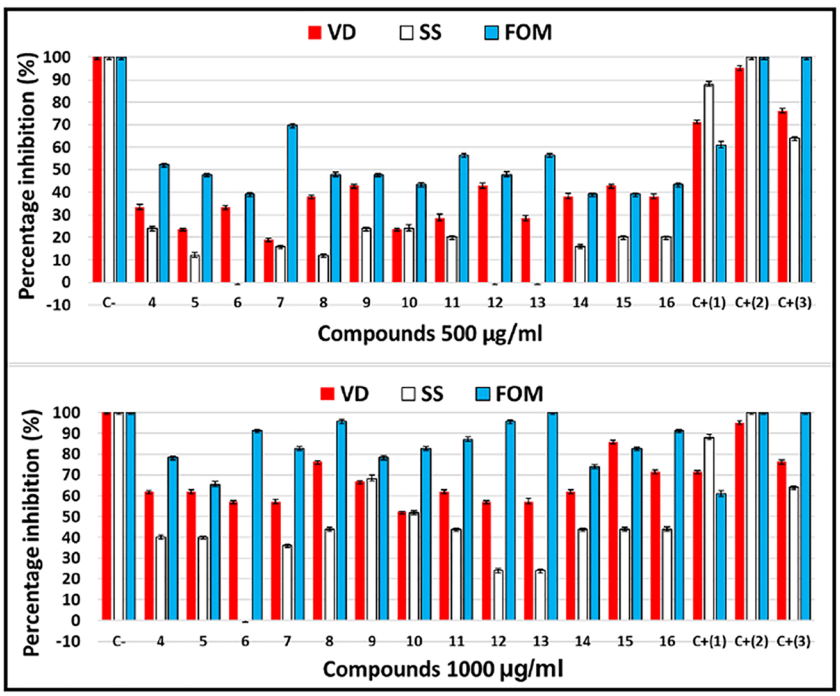


Figure 3. Percentage inhibition of compounds against *V. dahliae* (VD), *S. sclerotiorum* (SS), and *F. oxysporum melonis* (FOM) for 500 and 1000 µg/mL; C+: Manep (1), Thiram (2), and Captan (3) positive control; C−: negative control. According to negative control, mycelial growth was calculated percentage inhibition values. [Color figure can be viewed at wileyonlinelibrary.com]

Table 4

Docking scores and highest occupied molecular orbital (HOMO) energies of compounds **5**, **6**, and **8** and natural ligand ZBA.

Comp. no.	DScore (kcal/mol)	E_{HOMO} (eV)
ZBA	−8.5	−6.69
5	−7.4	−5.72
6	−7.1	−5.22
8	−7.4	−5.74

6, and **8** were the most active against the *M. fructigena* disease. Docking studies were performed for these three ligands. Table 4 shows the docking scores and HOMO energies of compounds **5**, **6**, and **8** and natural ligand ZBA; Figures 4–7 show docking studies for the active bonding zones of the target protein.

It was seen that the ligand bonded to the active bonding sites of receptors with weak, noncovalent interactions and more specifically with hydrogen bonding and alkyl interactions. As seen in Figure 4, the O atom of the receptor and the reference ZBA ligand formed hydrogen bonds with lengths of 2.49, 2.58, 2.17, and 2.82 Å with amino acids TYR413, THR366, HIS156i, and LEU365 of the receptor, respectively. N and F atoms of ligand **5** formed hydrogen bonds with lengths of 2.37 and 2.19 Å with TYR413 and MET161 of the receptor, respectively. All of the three ligands yielded values similar to the reference ligand (ZBA) according to their bonding affinities as shown in Table 4. In conclusion, it is possible to say that ligands **5**, **6**, and **8** could be candidate inhibitor molecules for the 2RKV target structure. Also, as seen in Table 4 and stated in the literature [39], high HOMO energy values resulted in high docking scores. The findings obtained in this study suggest that these compounds could be new potential inhibitors with biological activity for the 2RKV protein structure and used in *in vitro* studies.

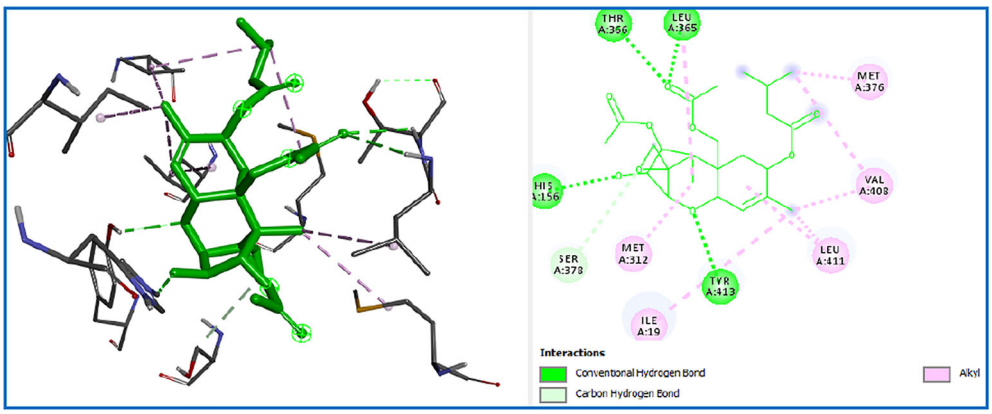


Figure 4. The 3D and 2D presentation of docking results of the ZBA ligand in the active region of the 2RKV protein. [Color figure can be viewed at wileyonlinelibrary.com]

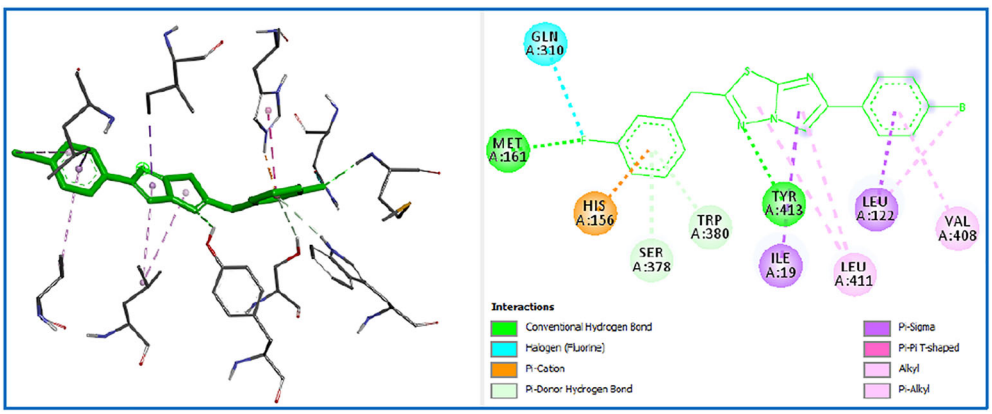


Figure 5. The 3D and 2D presentation of docking results of ligand **5** in the active region of the 2RKV protein. [Color figure can be viewed at wileyonlinelibrary.com]

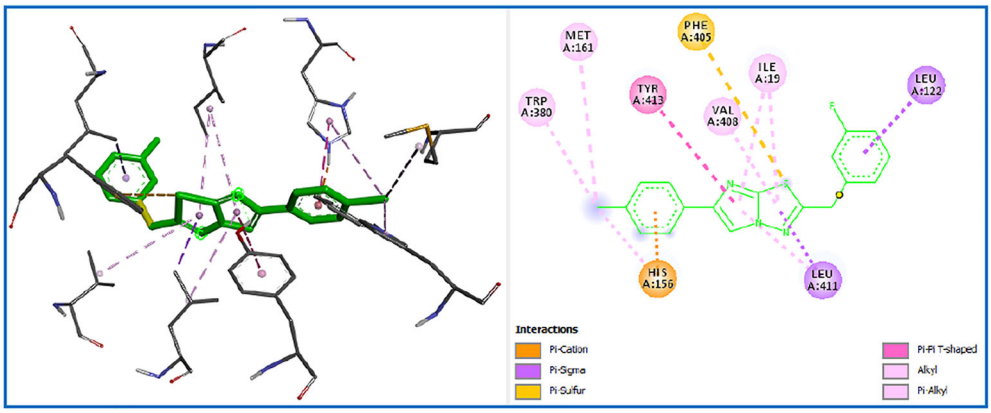


Figure 6. The 3D and 2D presentation of docking results of ligand **6** in the active region of the 2RKV protein. [Color figure can be viewed at wileyonlinelibrary.com]

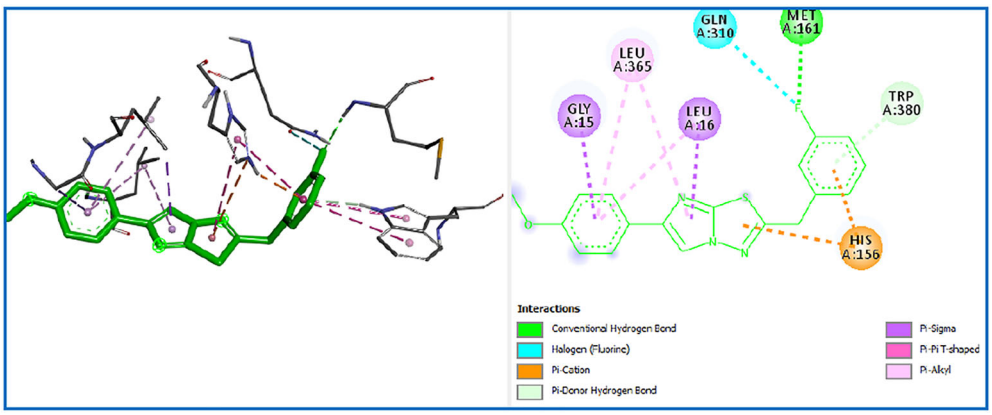


Figure 7. The 3D and 2D presentation of docking results of ligand **8** in the active region of the 2RKV protein. [Color figure can be viewed at wileyonlinelibrary.com]

CONCLUSIONS

Imidazo[2,1-*b*][1,3,4]thiadiazole derivatives, the target compounds of this study, were synthesized using simple reaction methodologies. All of the synthesized compounds were characterized using various analysis methods.

Biological activity tests were applied to the target compounds. According to the results obtained, all of the synthesized compounds showed moderate to high levels of antifungal activity against various plant pathogens.

After calculating the stable structures of the selected compounds (**5**, **6**, and **8**), docking simulation processes were used for HOMO–LUMO energies and possible bonding models and confirmations for the molecule. It was found that the HOMO energy values and affinity energy values of the compounds were proportional.

All of the findings obtained in this study indicate that these compounds could be new potential inhibitors for the 2RKV protein structure.

EXPERIMENTAL

The ^{1}H NMR and ^{13}C NMR spectra of the compounds were measured in DMSO-d_6 using an Agilent NMR VNMRS spectrometer at 400 and 100 MHz, respectively. Chemical shift values are given in ppm (δ) with tetramethylsilane as internal standard. The IR spectra were recorded in a Bruker Optics Alpha FTIR in attenuated total reflection (ATR). The mass spectra were measured with a Thermo TSQ Quantum Access Max LC-MS/MS spectrometer. Elemental analyses were performed on a LECO 932 CHNS (Leco-932, St. Joseph, MI, USA) instrument, and the results were within ±0.4% of the theoretical values. Melting points were recorded on a Thermo Scientific IA9000 series apparatus and were uncorrected. All of the chemicals were obtained from Sigma Aldrich Chemicals.

Computational details. Molecular docking studies were performed using the AutoDock Vina [42] and Discover Studio Visualizer 4.5 [43] programs. Optimized

structures and HOMO energy values of all ligands were calculated using the 6-32G(d) base set density functional theory (B3LYP) theory with the Gaussian 09 program.

Crystallographic analysis. X-ray diffraction data of crystals were collected with a Bruker AXS APEX CCD [45] diffractometer using MoKα beams. The crystal was kept at 293(2) K during data collection. Using Olex2 [46], the structure was solved with the ShelXT [47] structure solution program using direct methods and refined with the ShelXL [48] refinement package using least squares minimization.

Fungi culture. *M. fructigena* (isolated from apple in Gümüşhane, Turkey), *R. solani* (isolated from potato in Tokat, Turkey), *A. solani* (isolated from tomato in Antalya, Turkey), *V. dahliae* (isolated from tomato in Antalya, Turkey), *S. sclerotiorum* (isolated from cucumber in Antalya, Turkey), and *F. oxysporum* f.sp. *melonis* (isolated from melon in Hatay, Turkey) plant pathogens were used for *in vitro* antifungal activity study. The pathogens were grown on a potato dextrose agar medium at 22°C ± 2°C for about 7 days.

Synthesis. ***General procedure for the synthesis of 2-amino-1,3,4-thiadiazole derivatives (2a–b).*** Compounds **2a**–**b** were synthesized according to a method given in the literature [38,39].

In a round-bottomed flask, compounds (**1a**–**b**) (0.1 mol) and thiosemicarbazide (0.15 mol) in trifluoroacetic acid (50 mL) at 60°C were stirred for 3 h. The progress of the reaction was monitored by thin-layer chromatography at appropriate time intervals. After completion of the reaction, the mixture was poured into 500 mL of ice-cold water and neutralized with ammonia. The solution was filtered, and the solid matter was obtained. It was washed with deionized water, ethanol, and diethyl ether, respectively. The solid was recrystallized from the appropriate solvent. The physical properties and spectral data derived from the obtained products are listed below.

5-(3-Fluorobenzyl)-1,3,4-thiadiazol-2-amine ***(2a).*** White crystals, yield: 17.16 g (82%), mp 201–203°C (from DMF-EtOH, 1:4). IR (ATR, cm^{-1}): 3265–3114 ($-NH_2$), 3059 (Ar–CH), 2958 (Aliph. CH), 1604 (C═N). ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.16 (s, 2H, $-CH_2$), Phenyl–H [7.11–7.07 (m, 3H), 7.35 (q, J = 12.0 Hz, 1H)], 7.03 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 35.39 ($-CH_2$), Phenyl–C [114.001 (CH), 115.98 (CH), 125.27 (CH), 131.06 (CH), 141.29 (C), 163.83 (C)], Thiadiazole–C [157.12 (C), 169.33 (C)]. MS: *m/z* 209.82 (M^+, 100). *Anal*. Calcd. for $C_9H_8FN_3S$: C, 51.66; H, 3.85; N, 20.08. Found: C, 51.62; H, 3.78; N, 20.18.

5-(4-Nitrobenzyl)-1,3,4-thiadiazol-2-amine ***(2b).*** White crystals, yield: 18.66 g (79%), mp 181–182°C (from DMF-EtOH, 1:3). IR (ATR, cm^{-1}): 3271–3112 ($-NH_2$), 3067 (Ar–CH), 2954 (Aliph. CH), 1602 (C═N). ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.32 (s, 2H, $-CH_2$), Phenyl–H [7.54 (d, J = 8.0 Hz, 2H), 8.16 (d, J = 8.0 Hz, 2H)], 7.14 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 35.59 ($-CH_2$), Phenyl–C [124.42 (CH), 130.67 (CH), 146.54 (C), 147.08 (C)], Thiadiazole–C [156.39 (C), 169.78 (C)]. MS: *m/z* 237.06 (M+1, 100). *Anal*. Calcd. for $C_9H_8N_4O_2S$: C, 45.75; H, 3.41; N, 23.72. Found: C, 45.68; H, 3.35; N, 23.65.

General procedure for the synthesis of imidazo[2,1-b][1,3,4] thiadiazole derivatives (4–16). In a two-necked flask, 2-amino-1,3,4-thiadiazole derivatives (**2a**–**b**) (4 mmol) were dissolved in absolute ethanol (30 mL). 2-Bromoacetophenone derivatives (**3a**–**i**) (4 mmol) were dissolved in absolute ethanol (20 mL) and then added drop by drop to this solution at room temperature with the assistance of a dropping funnel. Then, the mixture was refluxed and stirred for 12–16 h. The progress of reaction was monitored by thin-layer chromatography at appropriate time intervals. The excess of solvent was removed under reduced pressure and neutralized by aqueous sodium carbonate (Na_2CO_3) solution. The solution was filtered and washed with deionized water. The solid matter was recrystallized from acetone. The synthesized compounds were dried with P_2O_5 in a vacuum oven. The physical properties and spectral data derived from the obtained products are listed below.

2-(3-Fluorobenzyl)-6-phenylimidazo[2,1-b][1,3,4] thiadiazole ***(4).*** Brownish crystals, yield 0.77 g (62%), mp 134–136°C (from Acetone). IR (ATR, cm^{-1}): 3112 (Ar–CH), 2922 (Aliph. CH), 1596 (C═N). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 4.46 (s, 2H, $-CH_2$), 3F–Ar–H [7.14 (t, J = 16.0 Hz, 1H), 7.25 (t, J = 16.0 Hz, 2H), 7.41 (q, J = 12.0 Hz, 1H)], Phenyl–H [7.24 (t, J = 12.0 Hz, 1H), 7.38 (t, J = 12.0 Hz, 2H), 7.83 (d, J = 8.0 Hz, 2H)], Imidazole–H [8.63 (s, 1H)]. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 36.85 ($-CH_2$), 3F–Ar–C [114.69 (CH), 116.58 (CH), 125.07 (CH), 131.23 (CH), 139.15 (C), 161.47 (C)], Phenyl–C [125.75 (CH), 127.71 (CH), 129.09 (CH), 134.33 (C)], Imidazole–C [110.77 (CH), 145.38 (C)], Thiadiazole–C [145.32 (C), 163.89 (C)]. MS: *m/z* 309.64 (M^+, 100). *Anal*. Calcd. for $C_{17}H_{12}FN_3S$: C, 66.00; H, 3.91; N, 13.58. Found: C, 65.95; H, 3.97; N, 13.50.

6-(4-Bromophenyl)-2-(3-fluorobenzyl)imidazo[2,1-b] [1,3,4]thiadiazole ***(5).*** Light yellow crystals, yield 1.01 g (65%), mp 156–157°C (from Acetone). IR (ATR, cm^{-1}): 3113 (Ar–CH), 2927 (Aliph. CH), 1595 (C═N). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 4.46 (s, 2H, $-CH_2$), 3F–Ar–H [7.13 (t, J = 16.0 Hz, 1H), 7.24 (t, J = 16.0 Hz, 2H), 7.40 (q, J = 12.0 Hz, 1H)], Phenyl–H [7.56 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 2H)], Imidazole–H

[8.68 (s, 1H)]. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 36.85 (–CH_2), 3F–Ar–C [114.70 (CH), 116.58 (CH), 125.75 (CH), 131.22 (CH), 139.08 (C), 161.47 (C)], Phenyl–C [120.57 (C), 127.04 (CH), 132.02 (C), 133.61 (CH)], Imidazole–C [111.22 (CH), 145.58 (C)], Thiadiazole–C [144.21 (C), 163.89 (C)]. MS: *m/z* 389.54 (M+1, 100). *Anal*. Calcd. for $C_{17}H_{11}BrFN_3S$: C, 52.59; H, 2.86; N, 10.82. Found: C, 52.49; H, 2.88; N, 10.89.

*6-(4-Chlorophenyl)-2-(3-fluorobenzyl)imidazo[2,1-b][1,3,4]thiadiazole (**6**).* Light yellow crystals, yield 0.98 g (71%), mp 155–157°C (from Acetone). IR (ATR, cm^{-1}): 3109 (Ar–CH), 2925 (Aliph. CH), 1596 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.46 (s, 2H, –CH_2), 3F–Ar–H [7.13 (t, *J* = 16.0 Hz, 1H), 7.24 (t, *J* = 16.0 Hz, 2H), 7.40 (q, *J* = 16.0 Hz, 1H)], Phenyl–H [7.43 (d, *J* = 8.0 Hz, 2H), 7.84 (d, *J* = 8.0 Hz, 2H)], Imidazole–H [8.67 (s, 1H)]. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 36.84 (–CH_2), 3F–Ar–C [114.70 (CH), 116.37 (CH), 125.75 (CH), 131.21 (CH), 139.09 (C), 161.47 (C)], Phenyl–C [126.72 (CH), 129.12 (CH), 132.05 (C), 133.25 (C)], Imidazole–C [111.19 (CH), 145.56 (C)], Thiadiazole–C [144.18 (C), 163.89 (C)]. MS: *m/z* 343.68 (M^+, 100). *Anal*. Calcd. for $C_{17}H_{11}ClFN_3S$: C, 59.39; H, 3.22; N, 12.22. Found: C, 59.48; 49; H, 3.27; N, 12.14.

*2-(3-Fluorobenzyl)-6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**7**).* White crystals, yield 0.84 g (64%), mp 148–150°C (from Acetone). IR (ATR, cm^{-1}): 3117 (Ar–CH), 2985 (Aliph. CH), 1587 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.45 (s, 2H, –CH_2), 3F–Ar–H [7.12 (t, *J* = 16.0 Hz, 1H), 7.25 (t, *J* = 12.0 Hz, 2H), 7.41 (q, *J* = 16.0 Hz, 1H)], Phenyl–H [7.19 (dd, *J* = 12.0, 6.0 Hz, 2H), 7.85 (dd, *J* = 12.0, 6.0 Hz, 2H)], Imidazole–H [8.60 (s, 1H)]. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 36.83 (–CH_2), 3F–Ar–C [114.68 (CH), 116.57 (CH), 125.73 (CH), 131.28 (CH), 139.11 (C), 161.47 (C)], Phenyl–C [115.84 (CH), 127.02 (C), 130.89 (CH), 163.15 (C)], Imidazole–C [110.61 (CH), 145.36 (C)], Thiadiazole–C [144.48 (C), 164.14 (C)]. MS: *m/z* 327.79 (M^+, 100). *Anal*. Calcd. for $C_{17}H_{11}F_2N_3S$: C, 62.37; H, 3.39; N, 12.84. Found: C, 62.31; H, 3.44; N, 12.93.

*2-(3-Fluorobenzyl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazole (**8**).* Orange crystals, yield 0.91 g (67%), mp 156–158°C (from Acetone). IR (ATR, cm^{-1}): 3123 (Ar–CH), 2926 (Aliph. CH), 1596 (C═N), 1099 (–OCH_3). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.75 (s, 3H, –OCH_3), 4.45 (s, 2H, –CH_2), 3F–Ar–H [7.14 (t, *J* = 16.0 Hz, 1H), 7.24 (t, *J* = 16.0 Hz, 2H), 7.41 (q, *J* = 12.0 Hz, 1H)], Phenyl–H [6.95 (d, *J* = 8.0 Hz, 2H), 7.74 (d, *J* = 8.0 Hz, 2H)], Imidazole–H [8.50 (s, 1H)]. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 36.82 (–CH_2), 55.57 (–OCH_3), 3F–Ar–C [114.52 (CH), 116.56 (CH), 125.73 (CH), 131.22 (CH), 139.21 (C), 161.47 (C)], Phenyl–C [114.88 (CH), 126.40 (C), 126.99 (CH), 159.11 (C)], Imidazole–C [109.61 (CH), 145.44 (C)], Thiadiazole–C [145.02 (C), 163.65 (C)]. MS: *m/z* 339.79 (M^+, 100). *Anal*. Calcd. for $C_{18}H_{14}FN_3OS$: C, 63.70; H, 4.16; N, 12.38. Found: C, 63.77; H, 4.07; N, 12.29.

*2-(3-Fluorobenzyl)-6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**9**).* Yellow crystals, yield 0.74 g (52%), mp 226–228°C (from Acetone). IR (ATR, cm^{-1}): 3122 (Ar–CH), 2925 (Aliph. CH), 1592 (C═N), 1514–1333 (–NO_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.49 (s, 2H, –CH_2), 3F–Ar–H [7.14 (t, *J* = 16.0 Hz, 1H), 7.26 (t, *J* = 16.0 Hz, 2H), 7.42 (q, *J* = 12.0 Hz, 1H)], Phenyl–H [8.08 (d, *J* = 8.0 Hz, 2H), 8.25 (d, *J* = 8.0 Hz, 2H)], Imidazole–H [8.93 (s, 1H)]. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 36.87 (–CH_2), 3F–Ar–C [114.75 (CH), 116.63 (CH), 125.67 (CH), 131.24 (CH), 138.98 (C), 161.47 (C)], Phenyl–C [124.67 (CH), 125.79 (CH), 138.90 (C), 146.53 (C)], Imidazole–C [113.52 (CH), 143.14 (C)], Thiadiazole–C [140.89 (C), 163.89 (C)]. MS: *m/z* 354.77 (M^+, 100). *Anal*. Calcd. for $C_{17}H_{11}FN_4O_2S$: C, 57.62; H, 3.13; N, 15.81. Found: C, 57.71; H, 3.12; N, 15.74.

*4-(2-(3-Fluorobenzyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzonitrile (**10**).* White crystals, yield 0.94 g (70%), mp 186–187°C (from Acetone). IR (ATR, cm^{-1}): 3137 (Ar–CH), 2920 (Aliph. CH), 2211 (C≡N), 1596 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.47 (s, 2H, –CH_2), 3F–Ar–H [7.13 (t, *J* = 16.0 Hz, 1H), 7.25 (t, *J* = 16.0 Hz, 2H), 7.41 (q, *J* = 12.0 Hz, 1H)], Phenyl–H [7.83 (d, *J* = 8.0 Hz, 2H), 8.00 (d, *J* = 8.0 Hz, 2H)], Imidazole–H [8.84 (s, 1H)]. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 36.87 (–CH_2), 119.48 (C≡N), 3F–Ar–C [114.73 (CH), 116.40 (CH), 125.52 (CH), 131.31 (CH), 138.98 (C), 161.46 (C)], Phenyl–C [125.79 (CH), 131.23 (CH), 133.17 (C), 138.83 (C)], Imidazole–C [109.72 (CH), 146.18 (C)], Thiadiazole–C [143.51 (C), 165.15 (C)]. MS: *m/z* 334.79 (M^+, 100). *Anal*. Calcd. for $C_{18}H_{11}FN_4S$: C, 64.66; H, 3.32; N, 16.76. Found: C, 64.58; H, 3.25; N, 16.69.

*6-([1,1'-Biphenyl]-4-yl)-2-(3-fluorobenzyl)imidazo[2,1-b][1,3,4]thiadiazole (**11**).* Light yellow crystals, yield 1.03 g (67%), mp 173–175°C (from Acetone). IR (ATR, cm^{-1}): 3122 (Ar–CH), 2923 (Aliph. CH), 1595 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.47 (s, 2H, –CH_2), 3F–Ar–H [7.15 (t, *J* = 16.0 Hz, 1H), 7.25 (t, *J* = 16.0 Hz, 2H), 7.34 (t, *J* = 12.0 Hz, 1H)], Phenyl–H [7.69 (d, *J* = 8.0 Hz, 2H), 7.92 (d, *J* = 8.0 Hz, 2H)], Phenyl–Phenyl–H [7.47–7.39 (m, 3H), 7.68 (d, *J* = 8.0 Hz, 2H)], Imidazole–H [8.69 (s, 1H)]. ^{13}C NMR (100 MHz,

DMSO-d_6, δ ppm): 36.87 (–CH_2), 3F–Ar–C [114.70 (CH), 116.59 (CH), 125.61 (CH), 131.24 (CH), 140.17 (C), 161.48 (C)], Phenyl–C [125.79 (CH), 127.85 (CH), 131.24 (CH), 139.16 (C)], Phenyl–Phenyl–C [125.76 (CH), 126.90 (CH), 127.35 (CH), 129.36 (C)], Imidazole–C [110.95 (CH), 145.47 (C)], Thiadiazole–C [145.04 (C), 164.21 (C)]. MS: *m/z* 385.89 (M^+, 100). *Anal*. Calcd. for $C_{23}H_{16}FN_3S$: C, 71.67; H, 4.18; N, 10.90. Found: C, 71.62; H, 4.14; N, 10.88.

2-(3-Fluorobenzyl)-6-(4-(naphthalen-2-yl)phenyl) imidazo[2,1-b][1,3,4]thiadiazole ***(12)****.* Brownish crystals, yield 1.18 g (68%), mp 146–148°C (from Acetone). IR (ATR, cm^{-1}): 3124 (Ar–CH), 2916 (Aliph. CH), 1587 (C═N). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.48 (s, 2H, –CH_2), 3F–Ar–H [7.15 (t, *J* = 16.0 Hz, 1H), 7.25 (t, *J* = 16.0 Hz, 2H), 7.43–7.39 (m, 1H)], Naphthyl–H [7.51–7.45 (m, 2H), 7.92–7.86 (m, 3H), 7.98 (d, *J* = 8.0 Hz, 1H), 8.37 (s, 1H)], Imidazole–H [8.75 (s, 1H)]. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 36.87 (–CH_2), 3F–Ar–C [114.72 (CH), 116.61 (CH), 125.77 (CH), 131.24 (CH), 139.06 (C), 161.48 (C)], Naphthyl–C [123.16 (CH), 123.86 (CH), 126.23 (CH), 126.89 (CH), 128.07 (CH), 128.36 (CH), 128.62 (CH), 131.81 (C), 132.81 (C), 133.68 (C)], Imidazole–C [111.32 (CH), 145.62 (C)], Thiadiazole–C [145.36 (C), 164.25 (C)]. MS: *m/z* 359.71 (M^+, 100). *Anal*. Calcd. for $C_{21}H_{14}FN_3S$: C, 70.18; H, 3.93; N, 11.69. Found: C, 70.12; H, 3.89; N, 11.66.

2-(4-Nitrobenzyl)-6-phenylimidazo[2,1-b][1,3,4] thiadiazole ***(13)****.* White solid, yield 0.82 g (61%), mp 230–231°C (from Acetone). IR (ATR, cm^{-1}): 3126 (Ar–CH), 2924 (Aliph. CH), 1599 (C═N), 1520–1336 (–NO_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.63 (s, 2H, –CH_2), *p*NO_2–Ar–H [7.69 (d, *J* = 8.0 Hz, 2H), 8.23 (d, *J* = 8.0 Hz, 2H)], Phenyl–H [7.25 (t, *J* = 16.0 Hz, 1H), 7.38 (t, *J* = 16.0 Hz, 2H) 7.83 (d, *J* = 8.0 Hz, 2H)], Imidazole–H [8.64 (s, 1H)]. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 36.81 (–CH_2), *p*NO_2–Ar–C [124.35 (CH), 130.99 (CH), 145.49 (C), 147.31 (C)], Phenyl–C [125.08 (CH), 127.74 (CH), 129.10 (CH), 134.29 (C)], Imidazole–C [110.82 (CH), 145.31 (C)], Thiadiazole–C [144.26 (C), 163.26 (C)]. MS: *m/z* 337.32 (M+1, 100). *Anal*. Calcd. for $C_{17}H_{12}N_4O_2S$: C, 60.70; H, 3.60; N, 16.66. Found: C, 60.61; H, 3.55; N, 16.61.

4-(2-(4-Nitrobenzyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl) benzonitrile ***(14)****.* Light yellow crystals, yield 0.93 g (64%), mp 223–225°C (from Acetone). IR (ATR, cm^{-1}): 3099 (Ar–CH), 2947 (Aliph. CH), 2221 (C≡N), 1606 (C═N), 1520–1349 (–NO_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.64 (s, 2H, –CH_2), *p*NO_2–Ar–H [7.68 (d, *J* = 8.0 Hz, 2H), 8.22 (d, *J* = 8.0 Hz, 2H)], Phenyl–H [7.84 (d, *J* = 8.0 Hz, 2H), 8.00 (d, *J* = 8.0 Hz, 2H)], Imidazole–H [8.86 (s, 1H)]. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 36.83 (–CH_2), 119.46 (C≡N), *p*NO_2–Ar–C [124.34 (CH), 131.02 (CH), 146.18 (C), 147.32 (C)], Phenyl–C [112.97 (C), 125.54 (CH), 133.21 (CH), 138.80 (C)], Imidazole–C [109.76 (CH), 144.10 (C)], Thiadiazole–C [143.62 (C), 164.30 (C)]. MS: *m/z* 384.84 (M^+ + Na). *Anal*. Calcd. for $C_{18}H_{11}N_5O_2S$: C, 59.82; H, 3.07; N, 19.38. Found: C, 59.88; H, 3.00; N, 19.30.

6-([1,1'-Biphenyl]-4-yl)-2-(4-nitrobenzyl)imidazo[2,1-b] [1,3,4]thiadiazole ***(15)****.* Light yellow crystals, yield 1.09 g (66%), mp 230–231°C (from Acetone). IR (ATR, cm^{-1}): 3109 (Ar–CH), 2950 (Aliph. CH), 1587 (C═N), 1509–1336 (–NO_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.64 (s, 2H, –CH_2), *p*NO_2–Ar–H [7.69 (d, *J* = 8.0 Hz, 2H), 8.23 (d, *J* = 8.0 Hz, 2H)], Phenyl–H [7.69 (d, *J* = 8.0 Hz, 2H), 7.92 (d, *J* = 8.0 Hz, 2H)], Phenyl–Phenyl–H [7.34 (t, *J* = 12.0 Hz, 1H), 7.44 (t, *J* = 12.0 Hz, 2H), 7.69 (d, *J* = 8.0 Hz, 2H)], Imidazole–H [8.70 (s, 1H)]. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 36.82 (–CH_2), *p*NO_2–Ar–C [124.35 (CH), 130.99 (CH), 145.45 (C), 147.30 (C)], Phenyl–C [127.86 (CH), 129.39 (CH), 133.44 (C), 139.34 (C)], Phenyl–Phenyl–C [125.62 (CH), 126.90 (CH), 127.36 (CH), 140.16 (C)], Imidazole–C [110.99 (CH), 145.13 (C)], Thiadiazole–C [144.26 (C), 163.31 (C)]. MS: *m/z* 412.84 (M^+, 100). *Anal*. Calcd. for $C_{23}H_{16}N_4O_2S$: C, 66.97; H, 3.91; N, 13.58. Found: C, 66.90; H, 3.84; N, 13.61.

6-(4-(Naphthalen-2-yl)phenyl)-2-(4-nitrobenzyl) imidazo[2,1-b][1,3,4]thiadiazole ***(16)****.* Yellow solid, yield 1.1 g (71%), mp 183–185°C (from Acetone). IR (ATR, cm^{-1}): 3108 (Ar–CH), 2939 (Aliph. CH), 1586 (C═N), 1510–1333 (–NO_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.65 (s, 2H, –CH_2), *p*NO_2–Ar–H [7.70 (d, *J* = 8.0 Hz, 2H), 8.24 (d, *J* = 8.0 Hz, 2H)], Naphthyl–H [7.51–7.44 (m, 2H), 7.93–7.87 (m, 3H), 7.98 (d, *J* = 8.0 Hz, 1H), 8.37 (s, 1H)], Imidazole–H [8.77 (s, 1H)]. ^{13}C NMR (100 MHz, 00 DMSO-d_6, δ ppm): 36.84 (–CH_2), *p*NO_2–Ar–C [124.36 (CH), 131.01 (CH), 145.62 (C), 147.32 (C)], Naphthyl–C [123.18 (CH), 123.85 (CH), 126.25 (CH), 126.90 (CH), 128.08 (CH), 128.36 (CH), 128.63 (CH), 131.77 (C), 132.83 (C), 133.66 (C)], Imidazole–C [111.37 (CH), 145.46 (C)], Thiadiazole–C [144.25 (C), 163.36 (C)]. MS: *m/z* 386.73 (M^+, 100). *Anal*. Calcd. for $C_{21}H_{14}N_4O_2S$: C, 65.27; H, 3.65; N, 14.50. Found: C, 65.21; H, 3.57; N, 14.41.

In vitro antifungal activity. Antifungal activities of the compounds were determined using the disc diffusion method [49]. The compounds were dissolved in DMSO. In a laminar flow cabin, Whatman No. 1 sterile filter paper discs (6 mm) were impregnated with 50 µL of the compounds (corresponding to 500 and 1000 µg/mL of

compounds) and allowed to dry at room temperature for 4 h [50]. The compound-impregnated paper discs were then placed in potato dextrose agar medium in 90-mm sterile petri plates. Discs (5 mm in diameter) of 7-day-old cultures of test fungi were inoculated on the centers of the petri plates. All fungi were incubated at 22°C ± 2°C. There was a distance of 25 mm between the mycelium discs and paper discs. Obtained inhibition zones were recorded. The experiment was set up with three replicates and repeated two times. As a negative control, only DMSO was added to the discs. In the positive control, 50% Manep (2000 μg/mL), 80% Thiram (3000 μg/mL), and 80% Captan (2000 μg/mL) were used against the test fungi at the recommended doses. All antifungal activity values were determined by measuring the inhibition zone distance between the pathogen and paper disc [39].

The PI was calculated according to the following formula [51]:

$$\%\text{Inhibition} = \text{Inhibition zone in treatment}/\text{Control} \times 100,$$

where "control" is the mycelial growth of the negative control.

Minimum inhibitory concentration, minimum fungicidal concentration, and lethal doses. The MICs and MFCs of the compounds were tested by the twofold serial dilution method. The test compounds were dissolved in DMSO to obtain 1000 μg/mL stock solutions. For the MIC and MFC assays, each compound was prepared in concentrations of 1000, 500, 250, 125, 62.5, and 31.25 μg/mL, and 50 μL of each concentration of the compound was transferred onto a paper disc. Then the same methods as described for the *in vitro* antifungal activity studies were applied. The MIC was defined as the lowest test concentration that allowed no detectable mycelium growth. The MFC was defined as the lowest test concentration that allowed no mycelium growth of the organism on agar [52]. In addition, LD_{50} values were calculated. Six different doses used in the calculation of the MICs were calculated using the results of the inhibition zones. LD estimates for LD_{50} were determined with the software Polo Plus (LeOra Software).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.